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Complex Structure of the Nudix D. Radiodurans Coenzyme A Hydrolase with a tri-Magnesium cluster.

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Beamline(s): X25

Introduction: Nudix hydrolases are widely distributed enzymes characterized by the highly conserved consensus sequence, **GX₅EX₇REUXEEXGU** (where U is usually Ile, Leu, or Val), the Nudix box. A large number of sequences containing the Nudix motif have been found in the genomes of species from all three kingdoms in organisms ranging in complexity from viruses to humans. To date, several tens of these proteins have been enzymatically characterized; their substrates include nucleoside triphosphates, co-enzymes, sugar nucleotides, and dinucleoside polyphosphates. The common feature of the substrates is the presence of a Nucleotide diphosphate bound to another group(x). The compounds are either potentially toxic or they are important cell signaling molecules, regulators, and metabolic intermediates such as ADP-ribose, NAD⁺, NADH, FAD⁺ and UDP-glucose whose concentrations require modulation during different states of the cell.

D. radiodurans is an organism known for its ability to withstand gamma-radiation fluxes 200-fold greater than those withstood by *E. Coli* and 20 times more resistant to ultraviolet radiation. This organism is even resistant to desiccation. The proposed rationale for this resistance to stress, is that the *D. radiodurans*' genome, has an abnormally large number (21) of Nudix family enzymes, since it was observed that the variation in the number of Nudix genes reflects adaptability, stress tolerance or metabolic stress. The product of gene DR1184 from *D. radiodurans* is a Nudix enzyme that has Coenzyme A pyrophosphatase activity (CoAase).

CoAase has been shown to hydrolyze Coenzyme A (CoA) and its derivatives with preference for oxidized disulfide CoA. It has been observed that eukaryotic CoA pyrophosphatases, which all prefer disulfide CoA as a substrate, are located in the peroxisome, strengthening the hypothesis that they regulate the concentration of CoA derivatives in an oxidizing environment.

Methods and Materials: Native Coenzyme A hydrolase crystals were soaked in 10mM MgCl₂. The binary complex of Coenzyme A and Mg⁺² was determined with data to 1.7Å resolution collected on a CCD detector (ADSC) at beamline X25 at NSLS, Brookhaven National Laboratory. Diffraction data, processed with Denzo and Scalepack, have an R_{sym} of 9%. The model was refined using the CNS suite with a residual target to R_{crys}=19% (R_{free}=24%).

Conclusions: CoA pyrophosphatases are characterized by a conserved motif, UPF0035, that occurs before the Nudix motif, and is postulated to be involved in the recognition of CoA. We have determined the 3-dimensional structure of the CoAase from *D. radiodurans* in complex with its divalent cation, magnesium, to a resolution of 1.7 Å. These structures represent the first step to understand the mechanism of CoA pyrophosphatase and to provide structural basis for the specificity for disulfide CoA.

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References:

- [1]. S.B. Gabelli, M.A. Bianchet, M.J. Bessman and L.M. Amzel. The structure of ADP-ribose pyrophosphatase reveals the structural basis for the versatility of the Nudix family. *Nature Structural Biology*, 8 (5) 467-472
- [2]. W.Xu, C.A. Dunn, M.J.Bessman. The Nudix hydrolases of *Deinococcus radiodurans*. *Mol Microbiol.* 2001 Jan;39(2):286-90.